

FURTHER OBSERVATIONS ON THE PRODUCTION OF CANINE HYSTERIA BY FLOUR TREATED WITH NITROGEN TRICHLORIDE (AGENE PROCESS)

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In an earlier paper (Mellanby, 1946) it was shown that wheaten flour, "improved" by the so-called "agene" process, which involves the treatment by NCl_3 , produced, when eaten by dogs, the condition known as canine hysteria, or, in the U.S.A., as running fits; untreated flour from the same grist produced no such fits, and when it replaced treated flour in the diet the fits stopped. Reference was made to the earlier work of Wagner and Elvehjem (1944), in which, following up an unpublished observation by Parry, they showed that commercial wheat gluten produced symptoms of hysteria in dogs with great rapidity and intensity. Previously Melnick and Cowgill (1937) had produced convulsive reactions in dogs on diets of high gliadin content. It followed that, if all these observations were true, then the gluten which Wagner and Elvehjem used in their work and the gliadin of Melnick and Cowgill had almost certainly been prepared from agenized flour. As stated in the paper referred to above (Mellanby, 1946), "obviously the next test to be made in regard to canine hysteria was to compare the effects of wheat gluten made from untreated and agenized flour respectively, and this is now being done." These tests had actually been made before the 1946 paper was published, and the results expected had been obtained. A film illustrating the effect of gluten from agenized and untreated flour on the production and cure of canine hysteria was shown at the Ministry of Health on Jan. 20, at Vanderbilt University on April 1, and at the International Physiological Congress in July of this year.

It seems desirable to put these facts on record, and the opportunity will also be taken of giving a brief account of one or two other observations in this field.

Action of Gluten Prepared from Agenized and Untreated Flour

In all the experiments described below the flour was agenized to the extent of 8 g. of NCl_3 to 280 lb. (127 kg.) of flour. This is an ordinary degree of treatment of flour used for human consumption.

In the earlier experiments gluten was prepared by making a dough with flour; the dough was put in muslin, and the starch washed out under cold tap-water. It was soon found that gluten made in this way from agenized flour, when added to the basal diet (see previous paper), produced typical nervous reactions, including epileptiform fits and other symptoms of canine hysteria. The amount of gluten added daily to the diet of the dogs represented about 400 g. of original flour. Under these conditions dogs developed hysteria in about four days. The same amount of gluten prepared from untreated flour of the same grist produced no such fits and, if it were allowed to replace gluten made from agenized flour in the diet of animals with canine hysteria, they recovered. Later it was found that washing the dough with warm water at a temperature of 40–45° C. had certain advantages, such as a great reduction in the time of washing out the starch and a reduction in loss of gluten sticking to the muslin. Gluten produced from untreated flour by the hot-water method, when substituted for that from agenized flour, also cured the affected animals.

It was found that the gluten contained a considerable amount of the original fat of the flour, and, as a technical method of testing whether flour has had the NCl_3 treatment is by determining the chlorine in the petrol-ether soluble portion (Kent-Jones and Herd, 1930), it seemed possible in the early days of this work, that the toxic effect of the flour was associated with the fat fraction. Agenized flour was therefore extracted with a solution of equal parts of ether and of alcohol. After removal of the ether and alcohol the soluble concentrate was taken up as a suspension in oil and added to the diet of dogs. It was found that this fat fraction produced no hysteria but that the fat-free flour was as toxic as the original whole flour. It can therefore be deduced from this that the toxicity of the gluten was not associated with its fat content.

Having traced the toxic action of the agenized flour to the gluten fraction of the dough, an effort was then made

to see whether the toxicity was associated with the gliadin or with glutelin. Gluten was prepared from treated flour and allowed to stand overnight with 65% alcohol at 37° C. It was then possible to mince the gluten into a granular mass; this was allowed to stand with 65% alcohol at a temperature of 75–80° C. under a reflex condenser for 48 hours. During this period three partial changes of the alcohol were made. After extraction the solution was centrifuged, since filtering was impossible, and the precipitate, insoluble in 65% alcohol, was regarded as predominantly glutelin, whereas the protein in the alcoholic extract was regarded as mostly gliadin. The alcohol was driven off from the gliadin fraction, and the residue was put into a pressure steamer and steamed for one and a half hours, after which it was minced and added to the diets. In the case of the glutelin fraction also the remaining alcohol was driven off and the residue treated in the same way as the gliadin before being added to the diet. The gliadin fraction produced typical hysterical outbursts when added to the diet of dogs, as did the glutelin fraction, but in the latter case the toxicity was definitely less than that of the gliadin. When the gliadin and glutelin fractions were added together to the diet the effect was similar to that produced by a corresponding amount of the original gluten, and severe hysteria and fits developed. It can probably be deduced from this experiment that the toxic substance producing canine hysteria was associated both with the alcohol-soluble (mainly gliadin) and with the alcohol-insoluble (mainly glutelin) fractions, that the gliadin fraction was more powerful in this respect than that of the glutelin, and that the chemical treatment had not destroyed any of the toxic substance. It is possible that the presence of this toxic factor in both fractions may have been due to the incomplete separation of the two proteins, but on the whole it is more probable that both proteins are involved in this chemical reaction.

Production of Hysteria in Ferrets by Agenized Flour

Reference was made in the former paper to the fact that the nervous symptoms had not been seen in rats fed on agenized flour; the same was found to be true for mice, but typical symptoms have been produced in ferrets whether fed on agenized flour itself or on gluten prepared from this flour: fat-extracted agenized flour is as potent as the flour itself, but the extracted fat has no effect. Unagenized flour from the same grists produces no abnormal symptoms, and when fed to affected ferrets the symptoms

disappear. In connexion with these hysterical and other nervous outbursts produced in ferrets by this means the following points may be noted :

1. The affected animals are more drowsy than usual and tend to sleep a lot.
2. They are more vicious when handled and bite much more freely than normal animals.
3. Ferrets develop real hysteria in the sense of charging round the cages and running into the cage sides.
4. They develop true epileptiform fits when severely affected.

On the whole, however, ferrets do not appear to be quite so susceptible to these nervous abnormalities as do dogs. On the other hand, they have the advantage that experimental work can be done with much smaller quantities of the toxic agent. .

Messrs. Wallace and Tiernan kindly lent the apparatus for agenzizing flour on a laboratory scale. The flour used in most of the experiments reported here was treated in the laboratory whereas that used in those described in the 1946 paper was done in the mill.

REFERENCES

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